

COMPOUNDS FOR CHANGING THE PHYSICAL PROPERTIES OF ICE AND METHODS OF USE THEREOF

ACKNOWLEDGEMENTS

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CROSS REFERENCE TO RELATED APPLICATIONS

10 This application claims the benefit of U.S. provisional application Serial No. 60/485,908, filed July 10, 2003. This application is hereby incorporated by this reference in its entirety for all of its teachings.

BACKGROUND

15 The changes to ice properties and microstructure due to water salinity and addition of inorganic ions to water are reasonably well understood (Wettlaufer, 1998; Eicken, 1998). It has been proposed to use a solid, water-insoluble polymer coating on particles to prevent coagulation and freezing of particle mixtures with the possible admixing of anti-freeze agents (Kestner and Stoldt, 1981; Roe and Polizzotti, 1992). The use of additional colligative freezing-point depressants such as salt solutions or
20 water soluble organic compounds such ethylene glycol to reduce the amount and strength of ice bonding has been disclosed. For example, U.S. Patent No. 4,426,409 to Roe discloses the use of a cationic polymer (gelatin or ammonium-based compounds) to enhance the retention of freezing-point depressants within an aggregation of coal particles at low temperatures. This technology, however, does not
25 rely on implicit properties of organic polymers in reducing macroscopic ice strength but rather uses polymers to reduce wash-out of water soluble freezing-point depressants. Furthermore, its application is limited to coal with moisture contents typically <5 %.

The embodiments described herein have not been previously considered and have a significant impact on ice microstructure and properties. Additionally, the microphysical principles underlying these effects have not been considered previously in this context and differ principally from those identified in previous research.

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SUMMARY

Described herein are compounds for changing the physical properties of ice and methods of use thereof.

The advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

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BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below. Like numbers represent the same elements throughout the figures.

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Figure 1 shows a schematic depiction of the deployment and distribution of a hydrocolloid emulsion in an application to reduce ice strength and stresses upon vessels, pilings and port installations.

Figure 2 shows the bulk ice salinity as a function of Xanthan gum concentration in parent water. Asterisks and solid dots denote first and second batch of experiments under different environmental conditions (growth rates). Vertical bars indicate the standard deviation of bulk salinities between three replicate samples. Figure 2B shows the effective segregation coefficient k_{eff} determined from experiments summarized in Figure 2A.

Figure 3 shows the segregation coefficient k_{eff} vs. ice growth rate for different experiments. Asterisks and dots denote first and second batch of experiments conducted at different growth rates. Vertical bars indicate standard deviation for set of three replicate samples. The solid line indicates the growth rate dependence and maximum value determined in laboratory experiments on artificial NaCl ice by Cox and Weeks (1975). The increase in segregation coefficients as a result of addition of Xanthan gum occurred at much lower growth rates than the maximum values observed for ordinary NaCl ice.

Figure 4 shows the pore microstructure of ice grown at different XG concentrations (A: 0 g l⁻¹, B: 0.01 g l⁻¹, C: 0.1 g l⁻¹, D: 0.5 g l⁻¹). The horizontal thin sections are 50 mm wide and were obtained at approximately 9 cm depth in the ice cover. They show the *in situ* pore distribution as evident from injection of a white contrast agent into pores. Ice salinities for the corresponding layers are A: 8.1 ‰, B: 8.2 ‰, C: 11.7 ‰, D: 19.4 ‰.

Figure 5 shows microphotographs of pore microstructure of ice identical to that in Figure 4 but lacking contrast agent. (A: 0 g l⁻¹, B: 0.01 g l⁻¹, C: 0.1 g l⁻¹, D: 0.5 g l⁻¹). The horizontal micrographs are 0.235 mm wide and were obtained at approximately 9 cm depth in the ice cover. Brine inclusions appear dark with ice transparent/light. Ice salinities for the corresponding layers are A: 8.1 ‰, B: 8.2 ‰, C: 11.7 ‰, D: 19.4 ‰.

Figure 6 depicts a demonstration of ductile failure and low yield strength of ice grown from water with XG concentration of 0.5 g l⁻¹ (ice temperature -4.6 to -6.2 °C). Originally intact ice (A) completely disintegrates upon effortless kneading by hand (B). Figure 6C illustrates reduction in ice tensile strength σ_t due to increase in porosity.

Figure 7 shows ice grown from deionized water containing 0, 0.5 and 1 g/l Xanthan gum (left to right).

Figure 8 shows the vertical thin section of freshwater ice containing no

Xanthan gum, photographed in plain transmitted light (left) and between crossed polarizers (right).

Figure 9 shows the vertical thin section of freshwater ice grown from a 0.1 g/l Xanthan gum solution, photographed in plain transmitted light (left) and between
5 crossed polarizers (right).

Figure 10 shows the vertical thin section of freshwater ice grown from a 1.0 g/l Xanthan gum solution, photographed in plain transmitted light (left) and between crossed polarizers (right).

Figure 11 shows the effective *in situ* permeability of sea ice containing
10 variable Xanthan gum concentrations frozen at -20 °C from Xanthan gum solutions and saltwater of a salinity of 30 ppt.

Figure 12 shows the functional relationship between Xanthan gum solutions in water prior to freezing and the resulting effect on bulk salinity (ion retention) in the ice.

15 Figure 13 shows the effect of Xanthan gum on ion retention. Illustrated is the ratio of Xanthan gum to salinity in the water below the ice and the resulting deviation of the respective ratio in the resulting grown ice samples.

Figure 14 shows the vertical profiles of bulk salinity and EPS concentrations of replicate ice cores taken from artificial sea ice frozen in seawater with EPS (17936
20 µg gluc equiv l-1 (SD9602, n=8)) and without culture water of *Melosira arctic*: (a) ice frozen directly; (b) translation of bulk salinity increase in (a) into effective fraction of habitable space at respective temperatures -2 °C to -30 °C according to (Cox, G. F. N., and W. F. Weeks, Equations for determining the gas and brine volumes in sea-ice samples, J. Glaciol., 29, 306-316, 1983); (c) Ice melted, mixed and refrozen; and (d)
25 Ice melted, heated to 100°C and then refrozen.

Figure 15 shows images of horizontal ice thin sections from the 5-cm ice horizon stained with contrast titanium dioxide and sea ice containing only sea salt (A)

and EPS from *Melosira arctica* (B). The width of the images are 20mm. Microphotographs of unstained horizontal thin sections of the same ice of (A) and (B) illustrating the different structure in brine inclusions between the ice samples are shown. Intra- (A.1) and inter- crystalline brine pores (A.2) in the control are absent in ice grown from culture water of *Melosira arctica* (B. 1-2). The width of the microscopic images is 324 μm . Images of horizontal ice thin sections from the 5-cm ice horizon of sea ice containing only sea salt (C) and Xanthan gum 10mg L⁻¹ (D). The widths are identical to those in Figures 15A and B.

Figure 16 shows the functional relation between EPS concentration in the water (A) and in the ice (B) and the resulting bulk salinity of the ice sheet. A. Effect of EPS concentration from *Xanthomonas campestris* (Δ) and *Melosira arctica* (O) on the bulk salinity of the ice sheet in 200 L seawater water. Error bars represent standard deviations of three replicate cores. B. Functional relation between EPS concentration retained in sea ice and its corresponding bulk salinity for bacteria EPS *Xanthomonas campestris* (Δ), *Colwellia psychrerythraea* a (\square) and diatom *Melosira arctica* repetitively frozen (\blacklozenge) and heat treated (\diamond) and a replication in a 200L ice tank (O). Included are ranges of EPS concentrations measured in the field and bulk salinities of the experimental control for EPS of *Melosira arctica*.

Figure 17 shows the vertical profiles of particulate and dissolved organic material in the lower 10 cm of fast ice collected off the coast of Barrow. (A) is the vertical profile of pigments Chl a (\blacklozenge) and Pheophytin (\square) including their respective ratio to one another (o); (b) is the vertical profiles of particulate nitrogen (\blacklozenge) and the ratio of carbon to nitrogen in particles $>0.7 \mu\text{m}$ (Δ); (c) is the vertical profile of pEPS measured with the methods PSA (\square) and AB (\blacklozenge) and their relation to POC (\square); and (d) is the vertical profile of dEPS measured with the method PSA (\square) and the fraction of pEPS of total EPS (\square).

Figure 18 shows a schematic summary of significant correlations of environmental variables (Ammonia NH₄; Nitrate NO₃; Nitrogendioxide NO₂;

Phosphat PO_4 ; total dissolved nitrogen TDN; Silicate $\text{SI}(\text{OH})_4$; Phaeophytin Pheo; Chlorophyll a Chl a ; total EPS tEPS; dissolved EPS dEPS; particulate organic carbon POC; carbon and nitrogen elementary composition of filtered particulate material p C/N) in the melted 1.8 cm vertically resolved ice-core sections of the lower 10 cm of
5 Chukchi Sea ice using Spearman rank correlation. Lines represent significant correlations (positive correlations solid, negative correlations dashed) included are correlation coefficients followed by significance levels.

Figure 19 shows the mass molecular fractions of total EPS precipitated in ethanol and separated via differences in density in a NaCl and CsCl gradient. The
10 black line represents averaged concentrations per collected sample fractions of three parallel runs, included in gray are standard deviation. Concentrations of EPS are given in absorption of light 490nm of the Phenol-Sulfur-glucose complex of prior hydrolyzed macromolecules.

Figure 20a shows the frequency distribution of pore cross sectional areas for
15 sea ice collected in sea ice of Elson Lagoon 2001(\square) and the Chukchi Sea (\square) and artificial sea ice containing only sea salt (O) and water of a culture of *Melosira arctica* (\diamond) (pores analyzed, $n > 700$). Figure 20b shows the frequency distribution of surface to area ratios of crosses sectional images of the same ice.

DETAILED DESCRIPTION

20 Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific compounds, synthetic methods, or uses as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

25 In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly

dictates otherwise. Thus, for example, reference to "a hydrocolloid" includes mixtures of two or more such compounds, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where
5 the event or circumstance occurs and instances where it does not. For example, the phrase "optionally contains an additive" means that an additive can or cannot be present.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another aspect
10 includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

15 References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by
20 weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is
25 included.

By "contacting" is meant an instance of exposure by close physical contact of at least one substance to another substance.

By "sufficient amount" is the amount needed to achieve the desired result or results.

Disclosed are compounds, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of
5 these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a number of different hydrocolloids and other additives are disclosed and discussed, each and every combination and permutation of the hydrocolloid and
10 additive are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E,
15 and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and
20 the example combination A-D. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each
25 such combination is specifically contemplated and should be considered disclosed.

I. Hydrocolloids

In one aspect, one or more hydrocolloids can be used to change the physical properties of ice. The term "hydrocolloid" as used herein is defined as hydrophilic polymers of vegetable, animal, microbial, or synthetic origin that contain one or more

hydroxyl groups. In one aspect, the hydrocolloid can be an exopolymer or a derivative thereof. The term "exopolymer" as used herein is defined as an organic polymer secreted into the environment by a aquatic or terrestrial microorganism. The term "exopolymer" also includes a capsule of the naturally-occurring polymer
5 intimately associated with the cell surface of the microorganism. The term "exopolymer" is also referred to as an exocellular polysaccharide or EPS. The exopolymer can be a single polymer or a mixture of two or more polymers. When the exopolymer contains a mixture of two or more polymers, the polymers can possess different functional groups and/or molecular weight. Any of the exopolymers
10 described herein can be chemically modified to produce a derivative of the naturally-occurring exopolymer.

In one aspect, the exopolymer is produced from a microorganism from the kingdom protista or monera. In one aspect, when the microorganism is from the kingdom protista, the microorganism can be from the phyla Acrasiomycota (cellular
15 slime molds), Chrysophyta (golden algae), Euglenophyta (euglenoids), Rhizopoda (amoebas), Actinopoda, Chytridomycota (chytrids), Foraminifera (forams), Rhodophyta (red algae), Apicomplexa, Ciliophora (ciliated protozoans), Myxomycota (plasmodian slime molds), Zoomastigophora (zooflagellates), Bacillariophyta (diatoms), Dinoflagellata (dinoflagellates), Chlorophyta (green algae), Oomycota
20 (water molds), Diplomonada (archezoa), or Phaeophyta (brown algae). In another aspect, when the microorganism is from the kingdom monera, the microorganism can be a cyanobacteria, an archaeobacteria, a methanogen, an extreme halophile, a thermoacidophile, or an eubacteria. In a further aspect, the exopolymer is produced from a microalga, a microphyte, an unicellular alga, or an eubacteria.

25 In another aspect, the exopolymers disclosed in U.S. Patent Nos. 6,727,081; 6,689,402; 6,436,680; 6,416,978; 6,344,346; and 6,027,925, which are incorporated by reference in their entirety, can be used herein.

In one aspect, the exopolymer is produced from the diatom *Melosira arctica*. *Melosira arctica* is a filamentous diatom that commonly occurs within and below the

Arctic ice pack and produces large amounts of exopolymers. Not wishing to be bound by theory, it is believed that *Melosira arctica* attaches to the underside of ice and forms meter-long chains.

In one aspect, the hydrocolloid can be the hydrocolloid comprises agar,
5 arabinoxylan, carrageenan, carboxymethylcellulose, cellulose, curdlan, gelatin, gelan, β -glucan, guar gum, gum Arabic, locust bean gum, pectin, or starch.

In one aspect, the hydrocolloid can be alginic acid or the salt thereof. Alginic acid is a polymer of mannuronic acid and guluronic acid, with the proportion of the two depending upon the type of brown algae. Alginic acid can be readily converted to
10 the corresponding salt using techniques known in the art. Examples of alginates useful herein include, but are not limited to, sodium, potassium, or calcium alginate. Alternatively, propylene glycol alginates are available from C.P. Kelco Company. In another aspect, the acetylated alginates disclosed in U.S. Patent No. 5,444,160, which is incorporated by reference in its entirety, can be used herein.

15 In another aspect, the hydrocolloid can be Xanthan gum or a derivative thereof. Xanthan gum is a heteropolysaccharide of high molecular weight, composed of D-glucose, D-mannose and D-glucuronate moieties in a molar ratio of 2:2:1, respectively. In one aspect, the Xanthan gum is produced by the bacterium *Xanthomonas campestris*, which compound and its preparation are fully described in
20 U.S. Patent No. 3,659,026, which is incorporated by reference in its entirety. Other Xanthan gums that can be useful in the methods described herein can be obtained from other known *Xanthomonas* bacteria such as, for example, *Xanthomonas carotata*, *Xanthomonas incanae*, *Xanthomonas begoniae*, *Xanthomonas malverum*, *Xanthomonas vesicatoria*, *Xanthomonas papavericola*, *Xanthomonas translucens*,
25 *Xanthomonas vasculorum*, and *Xanthomonas hederae*.

A derivative of a Xanthan gum includes, but is not limited to, non-pyruvylated, non-acetylated, and non-pyruvylated-non-acetylated Xanthan gums whether produced through fermentation of mutant strains of *Xanthomonas* or produced through chemical or enzymatic processes performed on conventional

Xanthan gum or any combination thereof. In one aspect, the derivative of the Xanthan gum can be prepared by fermentation of mutant strains of *Xanthomonas campestris* as described in U.S. Patent No. 5,514,791, the disclosure of which is incorporated herein by reference. Additionally, non-acetylated Xanthan gum may be prepared by chemical deacetylation of Xanthan gum produced by *Xanthomonas campestris* as described in U.S. Patent Nos. 3,000,790 and 3,054,689. Alternative methods for generating derivatives of Xanthan gum are disclosed in U.S. Patent Nos. 6,432,155; 6,432,359; 4,713,449; and 5,772,912, which are incorporated by reference in their entireties.

10 II. Methods of Use

Any hydrocolloid described herein can be used to change the physical properties of ice. The term "changed" as used herein is defined as increasing or decreasing a particular property of ice when contacted with one or more hydrocolloids when compared to ice that has not been contacted with the hydrocolloid(s). Various physical properties of the ice can be changed with the use of a hydrocolloid, which will be discussed below.

In one aspect, the hydrocolloid can reduce the tensile strength of ice when compared to ice that has not been contacted with or formed from the hydrocolloid. By reducing the tensile strength of ice, the resultant ice is not as hard and can be further manipulated or handled, which will be discussed below. The tensile strength of ice that has been contacted with or formed from the hydrocolloid will vary depending upon the selection of the hydrocolloid, the type of water that is used to produce the ice, the impurities or dissolved materials present in the ice and/or water, and environmental conditions. In one aspect, ice produced from one or more hydrocolloid(s) has a tensile strength of 0.3, 0.4., 0.5, 0.6, 0.7, 0.8., 0.9, 1.0 MPa (where any range can be formed from these values), or any combination endpoints lower than the tensile strength of ice that has not been contacted with or formed from the hydrocolloid. In another aspect, the tensile strength of ice formed by or contacted with the hydrocolloid is 10% to 90%, 20% to 90%, 20% to 60%, 30% to 70%, or 30%

to 60% lower than untreated ice.

Not wishing to be bound by theory, it is believed that the hydrocolloid can reduce tensile strength of ice by a number of different mechanisms. In one aspect, when the hydrocolloid is used to produce ice or is contacted with ice, the resulting ice is less permeable. This results in ice having a greater tendency of entrapping gasses and dissolved materials. In other words, the ability of gasses and other dissolved materials such as, for example, inorganic ions, salts, dyes, and organic particles, to diffuse from the ice at the ice-water interface is reduced. In one aspect, the salinity of ice treated with or formed by the hydrocolloid can be substantially higher (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 300%, 400%, or 500%) than ice not formed from or contacted with the hydrocolloid. The salinity of ice can also be expressed as a segregation coefficient, which is defined as the concentration of salt in the ice divided by the concentration of the salt in the bulk water. In one aspect, the segregation coefficient of ice formed from or contacted with the hydrocolloid is from 0.2 to 0.95, 0.3 to 0.95, 0.4 to 0.95, 0.5 to 0.95, 0.6 to 0.95, 0.7 to 0.95, 0.8 to 0.95, or 0.9 to 0.95.

In another aspect, when the ice retains dissolved materials and/or gasses, which is facilitated by the hydrocolloid, the morphology of the ice changes as well. Pore sizes of ice contacted with or formed by the hydrocolloid are significantly higher than pore sizes of untreated ice. In one aspect, the pore sizes in treated ice are 10%, 20%, 30%, 40%, 50%, 75%, 100%, 125%, 150%, 200%, 300%, 400%, or 500% greater than the pore size of ice not treated with the hydrocolloid.

In another aspect, when the water used to produce the ice is brine, the hydrocolloid can increase the viscosity of the brine, which can significantly reduce future desalination of the ice by the brine. Therefore, the more dissolved materials or gasses the ice can retain or the longer the dissolved materials or gasses are retained by the ice, the tensile strength of the ice can be reduced. The hydrocolloids can facilitate the retention of dissolved materials and gasses in the ice, which ultimately reduces the tensile strength of the ice.

In the case when ice formation has already occurred, the ice can be contacted with the hydrocolloid using a variety of techniques. Examples of such techniques include spraying, dipping, coating, or brushing the ice with the hydrocolloid. In another aspect, the hydrocolloid can be mixed with water prior to ice formation using techniques known in the art. The water can be fresh water, saline water, or water that contains other dissolved materials that can be entrapped by the ice. In another aspect, the hydrocolloid can be in the form of a foam or emulsion and used as an effective carrier of other components including, but not limited to, additives, stains, cryoprotectants, anti-fouling agents, anti-corrosion agents, and the like. The amount of the hydrocolloid used will also vary depending upon the hydrocolloid that is selected, the type of water, and the environmental conditions. In one aspect, the amount of hydrocolloid that can be used is greater than 0.05 g/L of water. In another aspect, the amount of the hydrocolloid is from 0.05 to 10g/L, 0.05 to 9 g/L, 0.05 to 8 g/L, 0.05 to 7 g/L, 0.05 to 6 g/L, 0.05 to 5 g/L, 0.1 to 5 g/L, or 0.2 to 5 g/L.

The use of hydrocolloids to change the physical properties of ice provides numerous advantages. First, hydrocolloids are commercially available or easily prepared and efficient at low concentrations at very low production costs (*e.g.*, Xanthan gum is commercially available at prices of around \$6.60 per kg). The hydrocolloids are for the most part environmentally degradable at a rate slow enough to persist for months during the cold season but fast enough to prevent build-up during the warm season. Due to their general high viscosity, the hydrocolloid will not disperse or be diluted in the environment. Finally, the hydrocolloid has a tendency to adhere to or concentrate at surfaces where its impact is greatest.

In one aspect, once the hydrocolloid is incorporated into the ice, the desired effect persists for long periods of time due to the greatly reduced desalination rates discussed above. Thus, there is no need for re-application of the hydrocolloid to the ice. Furthermore, the softened ice can still maintain (and even improve) its insulating properties with beneficial environmental and strategic effects.

There are numerous applications with respect to the use of hydrocolloids for

changing the physical properties of ice. In one aspect, it would be desirable to soften ice in the natural environment where engineering or industrial tasks are performed. For example, the introduction of hydrocolloid into surface waters at the onset of fall freeze-up and during winter ice growth in cold region harbors or other marine
5 installations can be performed. Such a procedure would allow for operations in these waterways during the course of winter without restrictions or damage due to solid ice. It would furthermore significantly reduce or abate potential damage due to build-up of ice internal stresses that are known to cause substantial, recurring damage to structures in important waterways. In one aspect, the hydrocolloid is Xanthan gum or
10 a hydrocolloid produced from *Melosira arctica*.

In this aspect, the advantages are numerous. First, the hydrocolloid does not prevent ice formation, which maintains the insulating properties of an ice cover (with better insulating properties due to entrainment of gas bubbles and reduced brine drainage). Thus, heat loss from a water body is actually minimized, which reduces
15 the total amount of ice formed while at the same time reduces moisture fluxes that would otherwise lead to fog and icing problems. Similarly, from a defense-strategic perspective, the application of the hydrocolloid would reduce ice strength while giving the appearance of port facilities being completely ice-locked. Second, in contrast with other additives that affect the freezing point of water through colligative
20 properties that require massive amounts of potentially harmful substances (Roe and Polizzotti, 1992), the hydrocolloid is highly effective even at very low concentrations (e.g., $<1 \text{ g l}^{-1}$, as compared to, e.g., tens to hundreds of grams per liter for glycol-based freezing-point depressants).

In one aspect, the hydrocolloid can be deployed as an emulsification of
25 seawater pumped *in situ* at the bottom or at mid-water level, mixed with air from an above-ground intake and dispensed through a rotating arm or series of mobile or fixed pipes (Figure 1). The air bubbles retained within the viscous fluid would induce the emulsion to float up under the ice and bring it into direct contact with the ice water interface, where its effect is strongest. Owing to the significant reduction in

molecular diffusivities of gases and dissolved matter in such emulsified water, additives as well as air bubbles can be maintained at higher concentrations for significant periods of time. Furthermore, build-up of the hydrocolloid under the ice in the laminar boundary layer further minimizes loss due to turbulent shear and advection. In other aspects, (1) the large-scale application of the hydrocolloid at oil and gas terminal installations in seasonally or episodically ice-covered water (e.g., Siberian gas tanker terminals) can be deployed either through surface spraying prior to freeze-up or injection of bubble-emulsion from below using rotary or mobile dispenser units; (2) the small-scale deployment of the hydrocolloid along quays, pilings, in lock chambers, marinas and other installations to reduce or abate build-up of stress against installations due to ice growth and ice pressure can be performed; and (3) the large-scale application of hydrocolloids in closed or semi-enclosed port basins, shipyard docks and other large marine enclosures, which will allow the passage of vessels without full or any ice-strengthening, can be utilized.

In another aspect, described herein are methods for reducing ice formation on an article by contacting the article with any hydrocolloid described herein. In this aspect, the article can be any article that is exposed to freezing temperatures and water, including, but not limited to, pipes, plumbing fixtures, airplanes, roadways, and the like. In this aspect, the hydrocolloid can be used a de-icing agent.

In another aspect, the hydrocolloid can be used as a lubricant under cold conditions. For example, Xanthan gum can be used as a drilling fluid additive, which reduces strain to drill gear when drilling in cold, hard ground such as permafrost. In another aspect, deployment of a hydrocolloid in combination with water/brine mixtures into ground surface or other semi-enclosed terrestrial structures can prevent or abate freeze-damage to buried or half buried structures in seasonally frozen or permafrost regions. In one aspect, adding a slurry of the hydrocolloid such as, for example, Xanthan gum and water, where the salinity of the water corresponds to the *in situ* soil salinity, can prevent freeze-damage of pipes and ensure access to buried structures even in the coldest part of the season while at the same time act as an *in situ*

lubricant.

In another aspect, the hydrocolloid can be used as a refrigerant in a cooling system. The phasing out of freon-based refrigerants has resulted in an increasing market for environmentally inert and harmless alternative refrigerants. One such technique widely employed is the use of ice-brine slurries for refrigeration applications ranging from food display cases in supermarkets to cooling of the railroad/car tunnel between the UK and France (Paul, 1993). The efficiency of the brine-ice coolant and the applicable temperature range increases with increasing ice content and increasing salinity. However, for commonly employed NaCl (and other inorganic salts) brines, the minimum temperature that can be achieved is limited by the temperature at which the freezing-point depressant properties of the dissolved salt in the brine are overtaken by the tendency for salt to precipitate out of solution. For NaCl solutions, this absolute limit corresponds to the eutectic at -21.2 °C (Spencer et al., 1990). A further limitation is the fact that ice-brine slurries can only be pumped up to a critical ice volume fraction (typically 10 to 50 %), which further limits the cooling efficiency. Not wishing to be bound by theory, by allowing the retention of greater amounts of salt in an ice-brine mixture, the hydrocolloid can reduce the likelihood of local stoppage and freeze-up of slurries even at higher ice volume fractions. At the same time, the reduction in strength of the ice slurry can be expected to greatly reduce or completely prevent the negative impacts of local freeze-up due to variations in temperature control (or allow for lower temperatures to be achieved in the coolant). Additionally, the rheological properties of the hydrocolloid (Groisman and Steinberg, 2001) concentrated in the remaining liquid will ease pumping and conveyance of the ice-liquid coolant. Ultimately, the efficiency of the cooling system will be increased (and maintenance decreased) when the refrigerant contains one or more hydrocolloids. Any cooling system that requires the use of a refrigerant can be used herein.

In another aspect, a hydrocolloid can be used in the food and agricultural industry. In one aspect, the hydrocolloid can be used in the softening and

modification of frozen food, which can alter the color, taste, and nutrition of the frozen food. Alternatively, by altering the crystal texture of ice, different forms of frozen food are contemplated (e.g., slurpee applications).

In another aspect, thermal and mechanical frost protection of agricultural
5 produce can be achieved by spraying a composition of the hydrocolloid onto fruit and crops sensitive to freeze damage. Not wishing to be bound by theory, it is believed that the hydrocolloid increases water retention on plants, reduces convective heat exchange, and reduces mechanical and dehydration damage from ice crystals present on the plant surface. In another aspect, fish and poultry dip-freezing in chilled brine
10 containing a hydrocolloid can be improved by achieving less dehydration of the meat while increasing chilling rate.

In another aspect, the hydrocolloid can be used to protect microbial species utilized in the food industry. In one aspect, extracellular cryoprotection and osmotic stabilization of yeasts, lactobacteria, and consumable microalgae can be achieved by
15 submerging the microbial species into a composition composed of water and a hydrocolloid and freezing the composition for storage and transportation.

In another aspect, described herein is a method for facilitating the removal of ice on an article, comprising contacting the article with a hydrocolloid prior to exposing the article to conditions that will promote ice formation on the article. In
20 one aspect, prior to freezing, when a food article is contacted with a hydrocolloid, the frozen food is preserved better and protected from freeze damage. For example, increased surface preservation, reduced dehydration, reduced mechanical damage, reduced phase segregation, and better mechanical separation are possible when the food article is contacted with hydrocolloid. In one aspect, the hydrocolloid can
25 protect the appearance of fish frozen in brine solution before shipment. By reducing the adhesive forces between the frozen fish, the fish scales and skin remain undamaged by the freezing/storage process.

In another aspect, the hydrocolloid can be used to treat sewage. Microbial

degradation of freshwater sewage at subzero temperatures is limited to a fraction of liquid water in the ice if sewage cannot be directly be pumped into the marine environment. Microbial activity is reported to temperatures below -8 °C. Sewage amended with a hydrocolloid and seawater can create the necessary habitat for
5 extended microbial degradation. Furthermore, because the hydrocolloid reduces the tensile strength of ice, the ice containing the sewage can be reworked and transported offsite.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in
10 the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and
15 deviations are possible. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the
20 product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions. Xanthum gum was purchased from Sigma Aldrich. The exopolymer produced by *Melosira arctica* was obtained by growing cultures enriched with f/2 nutrient medium fortified with silicate to high densities in 1,000 L of water illuminated over 24 hours
25 and sealed in polyethylene bags. The surface of the bag was covered by ice at an average temperature of around -2 °C. Cells were separated from the water by gravitational settling and the resulting water solution containing the exopolymer was used as is.

Example 1

Two sets of experiments were conducted by growing NaCl ice in four 200 l insulated tanks placed outside in Feb/March 2002 with ambient temperatures ranging between about -30 and -5 °C conducted under natural environmental conditions at the University of Alaska in Fairbanks. Xanthan gum was added to three of these tanks in each experiment at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5 and 1 g l⁻¹, and a control without any additive. A pressure relief mechanism prevented build-up of pressure within the tank during ice growth. Ice thickness was measured twice daily and the experiment terminated after an ice thickness of approximately 0.15 m was reached within a week. Three sets of replicate samples (10 x 10 cm in cross-section) were obtained from each tank and sectioned into 2.5 cm thick slices for measurements of salinity and XG concentration. Vertical and horizontal thin sections were cut and photographed as described by Eicken (1998).

An integral measure of the effect of Xanthan gum (XG) on ice growth and properties is shown in Figure 2a, which indicates the increase in bulk ice salinity as a function of XG concentration for a set of outdoor experiments. For XG concentrations of about 0.5 g l⁻¹ (in the parent water mass), salinity of the ice is increased by more than a factor of two. In terms of an effective segregation coefficient k_{eff} , describing the ratio between the salt concentration in the ice to that in the bulk water ($k_{\text{eff}} = S_i/S_w$), the values determined here range between about 0.24 and 0.85 at growth velocities of about 1 to 2.5 cm day⁻¹.

There are three important aspects to this finding. First, the segregation coefficient of 0.85 is the highest measured and reported so far (Figures 2b and 3) for any type of saline ice grown under natural conditions. At the same time, it is at least twice as high as values typically found in natural sea ice under rapid growth conditions. Second, while the segregation coefficients for control runs without XG additive are comparable to standard values (Weeks and Ackley, 1986), the addition of XG results in an increase in k_{eff} at growth velocities of up to an order of magnitude

lower than required for comparable effects without XG additive (Figure 3). As the method employed here to determine segregation coefficients is likely to underestimate the magnitude of k_{eff} to some degree, these values can be considered conservative estimates of the impact of XG on ice salinity. Third, as vertical salinity profiles and theoretical considerations demonstrate, the reduction in molecular diffusivities and the increase in brine viscosity due to the presence of XG (e.g., Marcotte et al., 2001) not only impact the segregation of salts at the ice-water interface but also drastically reduce later desalination of the ice, thus greatly prolonging the impact of the additive as compared to other additives which are continually being lost from the ice during aging.

The thin-section images shown in Figures 4 and 5 demonstrate that the ice undergoes profound changes in microstructure as well, with pore sizes and pore connectivity increasing substantially and further impacts on grain microstructure. Based on *in situ* ice temperature and salinity measurements, the *in situ* liquid (brine) pore volume fraction V_b/V was derived according to the method described by Cox and Weeks (1983). The brine porosity of ice grown from water with XG additive was higher by a factor of more than two. As corroborated by microstructural pore analysis, this increase in porosity represents a conservative estimate because the method of computing *in situ* brine volumes does not account for the additional impact of XG on the pore space. Furthermore, for very thin ice, differences in ice temperature due to different thickness also result in an underestimation of the increase in porosity under full-scale, natural conditions.

As ice porosity and microstructure are key parameters controlling the physical properties of sea ice on microscopic all the way to the largest of scales (Eicken, 2003), the addition of XG had a substantial impact on the properties and large-scale behavior of saline ice in different environments. One of the most pronounced impacts is on ice strength. Figure 6 highlights this by showing the low overall yield strength and highly ductile mode of failure of ice grown at XG concentrations of 0.5 g l^{-1} . In comparison with ordinary saline ice which can only be fragmented with great

difficulty, the ice grown from water with XG additive has the same large-scale morphology and plate appearance but is easily crushed to a slushy pulp.

While it will require dedicated measurements of ice strength using methods adapted to the different ice rheology, some measure of the impact on ice strength due solely to the increase in brine porosity can be assessed from computation of ice tensile strength σ_t based on a simple structural model, as described in detail by Cox and Weeks (1988). It needs to be pointed out, however, that this model assumes a “standard” microstructure and does not account for the significant reduction in ice strength due to the microstructural changes apparent in Figures 4 and 5, which also affect the mode of failure and significantly underestimates the impact of XG. Nevertheless, from Figure 6 it is apparent that the addition of 0.5 and 1 g XG/l⁻¹ liquid reduces ice strength (both the maximum value in the profile as well as the strength in the neutral fiber) by a factor of more than 2.

Example 2

In a pilot experiment, ice was grown in 1 l containers from deionized water with the addition of Xanthan gum at 0.5 and 1.0 l/g concentrations in two of these to assess impacts on ice properties. Figure 7 shows the results of this experiment, demonstrating the substantial increase in gas bubble retention (and smaller bubble sizes) along with the formation of intragranular, lamellar inclusions of polymers.

This pilot study was followed by a more detailed experiment. Freshwater ice was grown from deionized water by placing 5 l containers insulated at the sides and bottom and placed in an insulated larger container so as to only expose the water/ice surface in a cold room at ambient temperatures of -10 °C. Two of the containers contained 0.1 g/l and 1.0 g/l concentrations of Xanthan gum, while the third container was a control with no additions. The experiment was terminated after about 10 cm of ice had grown.

The ice grown without Xanthan gum exhibited the typical large columnar crystal sizes with layers of large cylindrical and ellipsoidal gas inclusions (Figure 8).

Adding Xanthan gum increased the number of gas inclusions (Figures 9 and 10) and resulted in a reduction of crystal size. At 1.0 g/l concentrations, some intragranular, lamellar inclusions, interpreted as polymer inclusions are visible. Furthermore, gas inclusions exhibit tilting and kinking, indicative of an impact of Xanthan gum on the growth of ice crystals at the ice-water interface.

Example 3

To test the effect of Xanthan gum on sea ice permeability, sea ice was grown in 1 meter long 10 cm diameter laterally insulated pipes filled with salt water and Xanthan gum solutions from the surface.

Sea ice was grown in a range of Xanthan gum between 0.01 to 1 g Xanthan gum/L containing solutions at a salinity of 30 ppt. Hydraulic conductivity of the ice with the underlying water was used to determine the sea ice permeability at in situ temperatures and conditions.

Results indicate that effective permeability of ice grown in Xanthan gum solutions is affected by one order of magnitude by Xanthan gum in a range between 0.001 to 1g per liter. However, effective permeabilities fell well within the range of natural sea ice between 10^{-9} and 10^{-8} m² with a detectable proportional increase in permeability with increasing Xanthan gum concentrations (Figure 11). This increase in permeability is partially attributable to the increase in sea ice porosity a result of increases in bulk salinity with increasing Xanthan gum concentration (Figure 12).

Segregation experiments indicate that Xanthan gum is proportionally segregated from the ice at a lower rate than ions above a concentration of 0.01 g Xanthan gum per liter. The effectiveness of Xanthan gum in retaining salt commences at 0.01g Xanthan gum per liter. This change in segregation behavior coincides with an effective increase in ion retention of the ice enriched with Xanthan gum above such concentrations (Figure 13). The ratio of Xanthan gum and salt in the underlying water however was not affected (Figure 13, linear regression).

Example 4**The effect of EPS containing culture water of *Melosira arctica* on sea ice properties**

The presence of *Melosira arctica* extracellular polymeric substances significantly influenced the properties of sea ice. An increase in the bulk salinity in ice containing EPS was detected repeatedly with increasing sea ice thickness during culture water frozen from *Melosira arctica* of a EPS concentration of 17936 $\mu\text{g gluc equiv l}^{-1}$ (SD9602, n=8) (Figure 14a) with EPS in the particulate fraction with only 1.52%. The overall final thickness of the ice accounted for 12.5-13 cm. Differences in bulk salinity between treatment and controls of vertical core sections of identical ice horizon ranged between 12.2 and 52.8 % and increased linearly with depth at a rate of 4.0 percent per centimeter ($r^2=0.97$). Repetition of the experiment after melting of the ice, stirring and re-freezing of the water (Figure 14c) confirmed the effect of microbial salt retention on sea ice. However, the linear relation of the salt segregation effect and depth of 1.9 percent per centimeter was less obvious ($r^2=0.56$). Heating the water containing EPS from *Melosira arctica* to 100°C prior to freezing, however, statistically significantly destroyed the microbial induced salt segregation effect.

Bulk salinity of the ice determines the volume fraction of inhabitable liquid space at isothermal conditions. Illustrating the effect of increased bulk salinity on the fraction of habitable space for the lowest portion of the ice (Figure 14b) at temperatures between -2 °C and -30 °C brine volume fractions were calculated according to (Cox and Weeks, 1983). The largest increase in brine volume occurred near the -2 °C isotherm resulting in a volumetric increase of brine from 40-60%.

Freezing rates were comparable between repetitive experiments and accounted for 0.146 cmh^{-1} $r^2=0.99$ (Figure 14a), 0.1278 cmh^{-1} $r^2=0.99$ (Figure 14b) and 0.1507 cmh^{-1} $r^2=0.99$ (Figure 14d). Bulk concentrations of EPS in the ice were highly variable and fluctuated between 4475 to 14473 $\mu\text{g gluc l}^{-1}$ and were not statistically

different between the three experiments.

A shift towards particulate EPS in form of gels (pEPS) ($>0.4\mu\text{m}$) was detected during the freezing process from initially 1.52% of pEPS of total EPS in the water to 4.9-9.8% in 9 replicate sections of identical ice horizon. Depth intervals had a pEPS fraction of; 9.8% (SD 5.5), 0-2.5cm; 9.0% (SD 2.5%), 2.5-5cm; 5.0% (SD 1.4%) 5-7.5cm; 7.4% (SD 5.5%) 7.5-10cm; 7.7% (SD 2.8%) 10-12.5cm. However, a moderate increase in pEPS fraction in the water during the experiments was also detected increasing from initially 1.52% ($n=3$) to 4.16%.

The effect of bacteria EPS, from *Colwellia psychrerythraea* and *Xanthomonas campestris*, on sea-ice properties.

Several benthic marine heterotrophic bacteria are prolific EPS producers and some of them can be found also in sea ice e.g. *Cytophaga* sp.. *Colwellia psychrerythraea* is a psychrophile benthic bacterium, which displayed an average cell specific total EPS production rate of $4.21 \cdot 10^{-8} \mu\text{g XGEQV l}^{-1} \text{d}^{-1}$ at -1°C based on 6 day averages during maximal growth in batch culture (Table 1). Seawater salinity (30 ppt) enriched with EPS from *Colwellia psychrerythraea* (concentration in water 7267 $\mu\text{g gluc l}$, SD 1062 7267 $\mu\text{g gluc l}$, $n=3$) displayed a statistically significant microbially induced increase in sea-ice bulk salinity, which was most prominent near the ice bottom and is similar to results obtained during the *Melosira arctica* experiment. Sea ice was collected at a total ice thickness of 5 cm both in treatment and control ice slabs, which resulted in a statistically significant increase in bulk salinity of the spiked ice by 1.7 ppt (t-Test: Two-Sample Assuming Equal Variances, two tailed, $n=12$). EPS concentrations in the water were 3.8 times higher than in the ice and similarly to observations in the *Melosira arctica* experiment at identical 2.5-5-cm ice horizon (only 1.06 factor difference). Results of this experiment showed that a natural marine bacterium can affect the properties of sea ice.

In a complementary experimental study, the effect of Xanthan gum was tested for its effect on physical properties of growing sea ice. Average bulk salinities of ice

core sections (n=3) increased with depth (Table 1). This effect was more prominent in ice frozen from water spiked with EPS. This experiment indicated that despite the terrestrial habitat of the tested bacteria, an effect on sea ice was observed pointing to an ubiquitous effect of EPS on ice growth dynamics.

- 5 Table 1. Experimental results of *Colwellia psychrerythraea* cell specific EPS production at -1°C and its effect on bulk salinity of artificially grown sea ice.

| Bacterial culture temperature | Specific total EPS production rate ($\mu\text{g XGEQV l}^{-1} \text{ d}^{-1}$) | Range |
|-------------------------------|--|---|
| -1°C | $4.21 \cdot 10^{-8}$ | $3.35 \cdot 10^{-8} - 7.54 \cdot 10^{-8}$ |
| Effect on sea ice: | Bulk salinity (salinity unit), (SD, n=11) | EPS conc. mg XGEQV l ⁻¹ , (SD, n=11) |
| Depth 1.25 cm | 9.0 (0.6) | 19.96 (9.78) |
| | 9.5 (0.7) | 26.52 (16.05) |
| | 10.5 (0.8) | 30.92 (22.65) |
| Depth 2.50 cm | 9.9 (0.8) | 9.81 (6.90) |
| | 10.0 (0.7) | 23.79 (17.69) |
| | 11.6 (0.8) | 37.33 (22.81) |

Table 1 Effect of Xanthan gum on ice-physical properties of bulk salinity in artificially grown sea ice in 200L outdoor ice tanks in comparison to effects of EPS induced by culture water of *Melosira arctica* displaying much slower ice growth rates.

5

| Experiment Ice Depth (cm) | Xanthan gum | | | | | | | | M200L Average |
|--|-------------|-----|---------|-----|---------|-----|---------|-----|------------------|
| | Average | SD | Average | SD | Average | SD | Average | SD | |
| 1.3 | 10.4 | 1.6 | 10.8 | 0.4 | 10.6 | 2.2 | 11.6 | 1.3 | 14.2 |
| 3.8 | 5.1 | 0.2 | 7.0 | 0.4 | 5.1 | 0.4 | 7.5 | 0.9 | 12.5 |
| 6.3 | 7.0 | 0.5 | 6.4 | 1.3 | 7.1 | 0.5 | 8.8 | 1.4 | 15.4 |
| 8.8 | 8.1 | 0.7 | 6.9 | 0.6 | 8.2 | 0.3 | 9.0 | 1.8 | 18.8 |
| 11.3 | 9.1 | 0.7 | 8.0 | 0.3 | 10.6 | 1.2 | 9.5 | 1.0 | - |
| 13.8 | 10.7 | 3.2 | 10.1 | 0.5 | 17.3 | - | 11.5 | 1.0 | - |
| 16.3 | - | - | 16.1 | 1.9 | - | - | 15.0 | 0.6 | - |
| Growth rate (a) ($r^2 > 0.90$) | 10.2 | | 10.1 | | 9.6 | | 8.2 | | 5.1 |
| EPS measured in the water (mg Gluc. EQVI-1) | 0.00 | | 0.31 | | 10.32 | | 194.11 | | 1.96 |
| Added conc. of XG to water mg l ⁻¹ | 0 | | 10 | | 100 | | 500 | | - |

The effect of EPS from *Melosira arctica* on brine inclusion structure of sea ice

EPS from *Melosira arctica* affected the macrostructure of brine inclusions qualitatively as illustrated in the micrographs of ice-thin sections contrasted with titanium dioxide (Figure 15). A change in the dimensions of pore and crystal lamellae structure in the presence of EPS resulted in a difference in qualitative textural appearance between ice containing only salt (A) and salt and spikes of EPS from the algae (B). Dark areas on the horizontal ice sections of the 5 cm ice horizon in Figures 15A and B indicate areas of ice crystals, which did not stain. White areas represent locations of formerly filled brine inclusions that were filled with stain. Overall visible brine inclusions were smaller but more numerous in ice containing EPS from *Melosira arctica* than in the control ice core. The effect of EPS from *Melosira arctica* resulted macroscopically in a different pore space structure than ice spiked with Xanthan gum of comparable EPS concentration (Figures 15C and D). Ice spiked with Xanthan gum displayed larger brine inclusions and outlined crystal lamellae.

On the microscopic scale, the difference was even more pronounced between control sea ice (Figure 15A.1-2) and EPS spiked sea ice (Figure 15B 1-2). EPS affected brine inclusions by altering their shape and orientation towards small and convoluted inclusions with anisotropic orientation and a high degree of apparent interconnectivity. Ice containing exclusively salt, in comparison, displayed brine inclusions with defined ellipsoid shapes and structural orientation. Due to the large complexity of shapes a distinction between intra- and inter- crystalline brine pores seen in the control ice (Figures 15A.1 and A2), it was impossible to discern in the presence of EPS from *Melosira arctica* (Figures 15B.1-2).

10 **Similarities and differences in the effect of EPS on sea ice bulk salinity by two fundamentally different organism classes**

Several factors influence the way an exopolymer can change the properties of ice. Factors include, but are not limited to, EPS concentration, EPS composition and the rate of ice growth. The results indicate that the concentrations of EPS in the water during freezing affected the bulk salinity of the ice regardless of the microbial origin of EPS. A functional relationship between EPS concentration and overall salt retention of the entire ice sheet could be determined using 200 L ice tanks (Figure 16a). This relationship appeared robust to the varying freezing rates among experiments. However, variability among replicates increased at high EPS concentrations (>0.75 g XGEQVI-1).

Though differences are not apparent for integrated ice core measurements in both EPS spiked experiments from *Melosira arctica* and *Xanthomonas campestris* of identical ice tank sizes (200L volume), they are different at their respective ice horizons. An increase in bulk salinity was observed when EPS spiked seawater (30 ppt) from *Melosira arctica* (concentration of 2188 µg gluc. equiv l-1 , SD 585µg gluc. equiv l-1, n=3) was frozen. The vertical salinity profile of the ice was comparable in shape to the profile of the EPS addition experiments of Xanthan gum despite significantly slower freezing rates during the *Melosira arctica* experiment. Ice

accretion over time was fitted to a logarithmic function $d = \log x$ resulting in a range of growth factors between 8.3-22.1 ($r^2 > 0.90$) in the Xanthan gum amended experiment and a factor of 5.1 in the *Melosira arctica* EPS amended experiment. Slower freezing rates should result in a higher segregation coefficient for salt. Minimum bulk salinities were higher in the ice being spiked with EPS from *Melosira arctica* (Figure 16b) and comparable to EPS concentration in other experiment. This result indicates a strong effect of *Melosira arctica* EPS, which is insufficiently resolved using integrated ice core measurements.

EPS from *Melosira arctica* has a high affinity for ice. During the *Melosira arctica* experiment, sea ice is relatively enriched in EPS in relation to the underlying water ($11855/3336 = 3.6 \mu\text{g gluc. equiv l}^{-1}$, $n=3$), whereas salt was lost with an enrichment factor of $15.2/43.4 = 0.35$. This stands in contrast to a lower ice enrichment for all Xanthan gum concentrations tested, which ranged between 0.6 to 1.1 and salt enrichment of the ice between 0.23-1.01 if EPS enrichment is normalized to bulk salinity.

To illustrate the differences in the effect of EPS on bulk salinity among different source organisms of *Xanthomonas campestris* (Xanthan gum), *Colwellia psychrerythraea* and *Melosira arctica* data were superimposed from different experiments onto Figure 16b). Untreated native EPS from *Melosira arctica* had a significantly stronger effect on bulk salinity (15.38, SD 0.84, $n=12$ cores) over those of bacteria origin. This effect was statistically significant (T-test unequal variances, $p < 0.001$) and accounted for an increase in the salt effect of 27.6 % over that of bacteria EPS, for similar EPS concentration (T-test assuming unequal variances, $p = 0.37$, $n=12$). Heat treated culture water of *Melosira arctica* clearly lost the effect on bulk salinity and fell into the functional relationship between bulk salinity and EPS concentrations (gXG EQIV l^{-1}) of $f(x) = 28.275x + 0.2911$, $r^2 = 0.92$ for *Xanthomonas campestris* and that of *Colwellia psychrerythraea*, (average bulk salinity 12.05, SD 1.15, $n=12$ cores).

The effect of EPS on melting rates of sea ice.

To test if Xanthan gum also had any effect on the melting process of ice, freely drifting ice pieces amended with and without EPS of *Xanthomonas*
 5 *campestris* were allowed to melt in seawater at an initial salinity of 30 ppt. During melting ice pieces were weighed in regular intervals and carefully placed back into the water. Water temperature increased steadily by $0.4^{\circ}\text{C day}^{-1}$ with a moderately faster rate during the first 0.5 days. The experiment was terminated when ice pieces lost approximately 30% of their initial weight and began to disintegrate. Both treatment
 10 and control ice pieces lost weight over time illustrated by the weight fractional reduction an indication of melting and brine loss. Melt rates were determined from fractional weight losses fitting average weight fractions of ice pieces to a linear function ($r^2 > 0.95$, $n=8$). Weight loss ranged between 10-18% by weight per day and differed by a factor of 1.77. Ice containing Xanthan gum of a concentration of 40 mg
 15 XG l^{-1} clearly affects the melting process by reducing its melting rate by 44 %.

Field observations

The results obtained during the ice tank experiments were related to natural sea-ice conditions by conducting a parallel study on the distribution and effect of EPS on the brine pore spaces. Phototrophic biomass was approximated by Chlorophyll *a*
 20 (Chl *a*), which ranged between 12.3 and 55 $\mu\text{g Chl } a \text{ l}^{-1}$ melted ice with a maximum in the lowest 1.8 cm of the ice sheet. Phaeophytin concentration (average 50.8, SD 34.4, $n=8$) was higher than Chl *a* concentration with an average Chl *a* to Pheophytin ratio of 0.44 (SD 0.01, $n=8$) pointing to a senescent sea-ice algae spring bloom (Figure 17a). EPS in the particulate fraction ($>0.4 \mu\text{m}$) was measured independently by two
 25 methods, the Phenol sulfuric acid method (PSA) and the Alcian blue dye-binding assay (AB). Using the PSA method, concentrations of pEPS were converted into carbon units using the conversions for glucose atomic weight carbon equivalents used during the calibration of the method. Concentrations were relatively uniform across

the lowest decimeter of the ice and accounted on average for an estimated $2.9 \times 10^{-4} \text{ g C l}^{-1}$ (SD $1.1 \times 10^{-4} \text{ g C l}^{-1}$). pEPS concentrations measured using the Alcian blue dye-binding assay were converted into carbon units using the conversion factor of Engel and Passow. Average concentrations reached $8.6 \times 10^{-4} \text{ g C l}^{-1}$ (SD $1.5 \times 10^{-4} \text{ g C l}^{-1}$).
 5 Both methods therefore differed on average by a factor of 2.9 with a higher estimate using the dye-binding assay.

Particulate organic carbon (POC, $>0.7 \mu\text{m}$), including some portions of pEPS, increased in the bottom 3 cm from $1.0 \times 10^{-3} \text{ g C l}^{-1}$ to $2.2 \times 10^{-3} \text{ g C l}^{-1}$ (average $1.33 \times 10^{-3} \text{ g C l}^{-1}$, SD $0.46 \times 10^{-3} \text{ g C l}^{-1}$ $n=8$) and was on average by a factor of 4.52 (PSA) and
 10 1.55 (AB) higher than pEPS (note the difference in filter size of $0.3 \mu\text{m}$).

Since the PSA method can also be applied towards measuring the dissolved EPS ($<0.4 \mu\text{m}$) fraction, dEPS concentration was measured and reached an average $8.1 \times 10^{-4} \text{ g C l}^{-1}$, SD $2.7 \times 10^{-4} \text{ g C l}^{-1}$. Concentrations did not vary significantly with depth. The concentration of dEPS was higher than that of pEPS, and was illustrated
 15 as a fraction of total EPS in Figure 17d. pEPS accounted on average for only 27.7% (SD 9.7) of the total EPS concentration and was independent of the distance from the ice water interface. The elementary composition of particulate organic material in carbon and nitrogen can be an indication of the relative freshness of the material assuming that growth limiting elements are remineralized at a faster rate. C to N
 20 ratios, however, changed little with depth and had an average value of 8.09 (SD 0.51). Average particulate nitrogen conc. accounted for $1.6 \times 10^{-4} \text{ g l}^{-1}$ (SD $0.65 \times 10^{-4} \text{ g N l}^{-1}$) and increased in the lower 3 cm of the ice following the small increase in Chl a (Figure 17a) concentration.

Macronutrient profiles in the bottom 10 cm (Table 2) of sea ice ranged for
 25 phosphate between 0.16 to $3.9 \mu\text{M}$ (average, 0.24 SD 0.07), for silicate between 0.25 to 1.37 (average 0.89, SD 0.34), for nitrate between 0.15 to 1.03 (average 0.32, SD 0.30), for nitrite below $0.06 \mu\text{M}$ and ammonia between 2.38 to $9.68 \mu\text{M}$ (average 4.94, SD 2.76). While phosphate and nitrite concentrations were uniformly

distributed with depth, concentrations in silicate dropped between the ice water interface and the 147.6 cm depth horizon by 82%, nitrate decreased also by 85%. In contrast, ammonia significantly increased towards the interior of the ice with a factor of 3.6. The ratios of total dissolved nitrogen to phosphate is an indication of a relative shift in the Redfield ratio of cells and respective nutrient limitations of the system leading to nutrient regeneration. Ratios increased from 10.6 to 34.5 with distance from the interface, which indicates a relative limitation of available phosphate compounds. The degree of a microbially nutrient regenerated system is also indicated in the ratio of nitrate to total dissolved nitrogen. The opposite trend of the ratio with depth to that of the Redfield ratio indicates a qualitative transformation of dissolved oxygenated nitrogen compounds to reduced compounds in the bottom ice by the microbial community, which suggests pronounced heterotrophic activity.

Table 2. Macro nutrient profiles of melted 1.8 cm ice-core sections of the lower most 10 cm of Elson Lagoon ice collected in June 2001. Included are ratios of Nitrogen to phosphate and Nitrate to total dissolved nitrogen compounds (Nitrate, Nitrite and Ammonia) as indicator of relative availability of macro nutrients for the microbial
5 bottom ice community.

| Depth (cm) | PO ₄ | Si (OH) ₄ | NO ₃ | NO ₂ | NH ₄ | N/P | NO ₃ /tot N |
|------------|-----------------|----------------------|-----------------|-----------------|-----------------|------|------------------------|
| 147.6 | 0.29 | 0.25 | 0.15 | 0.05 | 9.68 | 34.5 | 0.01 |
| 148.9 | 0.18 | 0.64 | 0.15 | 0.05 | 8.72 | 50.5 | 0.02 |
| 150.1 | 0.20 | 0.93 | 0.15 | 0.05 | 5.12 | 26.7 | 0.03 |
| 151.4 | 0.16 | 0.82 | 0.15 | 0.04 | 3.64 | 23.5 | 0.04 |
| 152.6 | 0.23 | 1.20 | 0.24 | 0.05 | 3.85 | 17.7 | 0.06 |
| 153.9 | 0.20 | 1.00 | 0.37 | 0.05 | 3.48 | 19.1 | 0.09 |
| 155.1 | 0.28 | 0.89 | 0.37 | 0.05 | 2.38 | 10.0 | 0.13 |
| 156.4 | 0.36 | 1.37 | 1.03 | 0.05 | 2.69 | 10.6 | 0.27 |

To graphically illustrate the interdependence of environmental variables that were present during our analysis, explorative statistical analysis of variables in the
10 bottom ice section were performed using Spearman correlations (Figure 18). Correlations between paired variables illustrated a strong spatial dependence of the compounds of nitrogen, silicate, and photopigments with distance to the water interface. Nitrate significantly correlated with 6 other variables and was located at a central position to other variables, illustrating its importance in fueling the sea ice
15 microbial community. Ammonia with 5 significant correlation partners was also located at a central position. The distinct shift from a nitrate (new production) to an ammonia dominated (microbially regenerated) system is again indicated in the predominantly positive correlation of nitrate with all variable partners and a predominantly negative correlation of ammonia with pigment concentrations and
20 depth. Phosphate, in contrast, correlated only positively with particulate nitrogen, which can be an indicator of fresh particulate material.

A positive correlation of total EPS (tEPS) with particulate nitrogen but a negative correlation to total dissolved nitrogen compounds relate tEPS close to

particulate biomass. Qualitatively tEPS concentrations followed closer a biomass of low C:N ratio, which can be an indication of healthy cells. However, no vertical trend of EPS in the bottom ice was observed allowing to link EPS directly to phototrophic biomass.

5 A description of EPS mass molecular composition was qualitatively determined by performing a mass molecular fractionation of ethanol-precipitated total EPS from melted parallel 10-cm ice-core segments from a nearby location (Elson Lagoon, Alaska). Mass molecular fractions of tEPS illustrated a diverse range of molecular weight classes (Figure 19). Several dominant mass molecular classes
10 appear at 3.0, 8.7, 54 and 183 kD with a weak shoulder at 16kD. tEPS is expressed in sugar equivalents, with concentrations of large molecules relatively emphasized. An unresolved remainder of total EPS in the molecular mass fraction >336 kD was present. Standard deviations of three replicate runs were largest around 8.7kD.

 Qualitative *in situ* microscopic observations of Alcian blue-stained EPS
15 aggregates inside ice thin core sections illustrated their high abundance near the ice water interface (Figure 19A-F, microscopic analysis at -10°C, 147.6 cm depth horizon). Alcian blue stains pEPS. At intermediate magnification, stained pEPS appears to be present in most of the pores. An analysis of 834 images taken from ice indicated that despite a large fraction (47.8%) of unstained pores (pores containing no
20 EPS or not reached by the stain) a significant fraction of pores (52.1%) contained stained pEPS (Table 3). The staining process did not appear to entrain air since 48.2 % of the pores contained no air bubbles followed by 19.3 % of the pores filled with small air bubbles displacing 25% the brine by area. Pores completely filled with air were very rare and occurred in only 1.6%. Brine inclusions not filled with stained
25 EPS (or not reached by stain) contained in 0.5% of the observed diatoms, and 2.9% detritus were in 27.0% very pointy in shape. Detritus concentrations were significantly higher than diatom concentrations in each of the pore categories, which is consistent with the low Chl *a* to Pheophytin ratio in melted ice cores supporting a senescent algal bloom. A clear increase in pointiness (53%) of pores was observed in

brine inclusions that were filled to 100% with pEPS. Qualitative images of diatoms either unicellular or as part of chains were always surrounded with stainable EPS matrix lining the cell surfaces. In many incidences, EPS appeared to cushion the chains with its matrix into the brine channel passages (Figure 19C). pEPS also
 5 occurred in pores at significant concentrations that were diatom free (Figure 20B, Table 3).

Table 3. Relative distribution of Alcian-blue stained pEPS and air inclusions inside brine inclusions of the 147.6 cm depth horizon. Information on co-occurrence of diatoms, detritus and pointy pore features of the brine inclusions in a total of 834
 10 inclusions are included.

| Area cover of inclusion by: | Relative frequency: | | Co-occurrence in same pore: | | |
|-----------------------------|---------------------|--------|-----------------------------|----------|--------------|
| | air bubble | pEPS | diatoms | detritus | pointy pores |
| 0% | 48.2 % | 47.8 % | 0.5 % | 2.9 % | 27.0 % |
| 25% | 19.3 % | 15.7 % | 5.3 % | 15.2 % | 43.0 % |
| 50% | 3.8 % | 8.5 % | 2.8 % | 16.7 % | 46.0 % |
| 75% | 0.7 % | 9.7 % | 3.7 % | 18.5 % | 47.0 % |
| 100% | 1.6 % | 18.2 % | 3.7 % | 14.5 % | 53.0 % |

An investigation as to whether EPS in a natural sea ice system leaves a detectable signature analogous to observations from the experimental ice-tank setting
 15 were microscopically analyzed and quantitatively described by complexity of pore space structure. The frequency spectrum of pore-space dimensions from experimental and natural sea ice illustrate different size distributions between ice spiked with EPS from *Melosira arctica*, artificial control sea ice, and natural sea ice at the 147.6 cm depth horizon. While the spectrum of pores of control sea ice and field samples
 20 displayed unimodal distributions of the spectrum of cross sectional areas, sea ice grown from water containing EPS of *Melosira arctica* had a distinct bimodal distribution with maxima at $6.3 \cdot 10^5 \mu\text{m}^2$ and $2.5 \cdot 10^9 \mu\text{m}^2$. The maximum of larger pores was significantly shifted towards larger pores compared to its control counterpart, with a maximum at $1.0 \cdot 10^7 \mu\text{m}^2$. This shift of a subclass of pores towards

increased numbers of larger pore areas point to a relative increase in volumetric dominance of larger pores in EPS manipulated sea ice. Also the complexity of pores suggests a higher degree of inter-connectiveness which is lost during a 2 dimensional analysis.

5 Natural sea ice collected of the coast of Barrow was similar in its spectrum of pore cross sectional areas to the control experimental sea ice revealing no influence of EPS on sea ice (Figure 20a). The frequency spectra in the surface to volume ratios (aspect ratio) of pores is a more sensitive means for detecting size specific structural differences. Experimental sea ice frozen from sea water containing EPS from
10 *Melosira arctica* displayed a distinct increase in relative surface area in 44% of the pores (Figure 20b) which had its maxima above that of the control ice. Natural sea ice showed only a small indentation in the tip of an otherwise unimodal distribution. Sea ice collected from Elson Lagoon and experimental control sea ice were uniformly distributed with their peaks offset to one another by a factor of 7. The experiments
15 described above were insufficient to resolve the observed differences between the pore dimensions of natural sea ice and the experimental sea ice.

Example 5

Five different treatments were performed on 0.7 L *Melosira arctica* culture water samples to determine the molecular nature of the active substance affecting the
20 physical properties of sea ice. Prior to freezing, samples from a *Melosira arctica* batch culture were treated according to the treatment scheme outlined in Table 4. To assure identical conditions, samples were placed in a thermo-insulated water bath 30 x 40 x 20 cm of a salinity of 33 ppt so that the water levels in the samples matched the water level of the surrounding water. Freezing of the samples and the surrounding
25 water was induced from the surface in a cold room set to -10°C and continued for approx 24 hours.

Treatments of Glycoprotein:

To knock out a potential glycoprotein, a denaturing buffer (0.5% SDS, 1% b-

mercaptoethanol) was introduced to the sample and incubated for 10 min at 37 °C. 1X G7 Reaction Buffer [50 mM Sodium Phosphate (pH 7.5 @ 25°C)], supplemented with 1% NP-40, at 37°C was also added.

To assure a complete deactivation of proteins, the enzyme N-Glycosidase F, also known as PNGase F (glycerol free), an amidase, was added to the sample, which cleaves glycoproteins between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from N-linked glycoproteins.

A control for the increase in temperature to 37°C was introduced. One additional heat treatment at 72 °C was performed to determine the sensitivity of the active agent to heat below 100°C. Data were compared to known conditions: *Melosira arctica* culture water untreated (temperatures <4°C) and a pure NaCl solution.

Table 4. Treatment conditions and resulting effects on sea ice microstructural features qualitatively assessed on repetitive photomicrographs at 250 x magnification.

| Treatments of <i>Melosira arctica</i> culture water (35ppt) | Pore space spatial complexity | | |
|---|-------------------------------|--------|------|
| | low | medium | high |
| Untreated | | | X |
| Denature and hydrolysis of glycoprotein at 37°C | | X | |
| Control for 37°C treatment | | | X |
| Heat treatment 72°C | | | X |
| NaCl 35ppt | X | | |

The results indicate that the effect on ice micropore structure was reduced but not completely lost in the Glycoprotein knock-out experiment. Other treatments had no effect. Earlier heat treatment experiments using *Melosira arctica* culture water to 100 °C had shown that the effect of lowered ion segregation was lost. These results

indicate that the glycoprotein(s) or protein in question are stable to temperatures of up to 72°C.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by
5 reference into this application in order to more fully describe the compounds, compositions and methods described herein.

Various modifications and variations can be made to the compounds, compositions and methods described herein. Other aspects of the compounds, compositions and methods described herein will be apparent from consideration of the
10 specification and practice of the compounds, compositions and methods disclosed herein. It is intended that the specification and examples be considered as exemplary.

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